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(54) Title: **METHODS OF USE OF THE ENZYMES OF MYCOTHIOI SYNTHESIS**(57) Abstract: The present invention utilizes three families of bacterial enzymes, which play a key role in mycothiol biosynthesis. The three families are bacterial cysteine:glucosaminyl inositol ligases (MshC) with catalytic ligase activity for ligation of glucosaminyl inositol and cysteine, bacterial acetyl-CoA:Cys-GlcN-Ins acetyltransferases (MshD) with catalytic activity for addition of an acetyl group to Cys-GlcN-Ins and bacterial MshA glycosyltransferase with catalytic activity for production of GlcNAc-Ins. The invention provides methods for using the mycothiol biosynthesis ligases, acetyltransferases or glycosyltransferases in drug screening assays to determine compounds that inhibit activity. The invention provides for treatment of actinomycete infections in mammals using antibiotics that inhibit production or activity of the enzymes of mycothiol biosynthesis, in particular MshC, MshD or MshA, and thereby reduce the production of mycothiol and the virulence of the infecting bacteria. Additionally, the invention provides a live mutant with a genome containing a modification in an endogenous enzyme of mycothiol biosynthesis gene. The invention also provides an expression vector comprising polynucleotides of *mshA*, *mshB*, *mshC* and *mshD*.